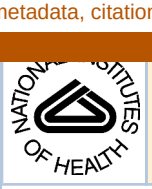



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# Using death to one's advantage: HIV modulation of apoptosis

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## Abstract

Infection by human immunodeficiency virus (HIV) is associated with an early immune dysfunction and progressive destruction of $CD4^+$ T lymphocytes. This progressive disappearance of T cells leads to a lack of immune control of HIV replication and to the development of immune deficiency resulting in the increased occurrence of opportunistic infections associated with acquired immune deficiency syndrome (AIDS). The HIV-induced, premature destruction of lymphocytes is associated with the continuous production of HIV viral proteins that modulate apoptotic pathways. The viral proteins, such as Tat, Env, and Nef, are associated with chronic immune activation and the continuous induction of apoptotic factors. Viral protein expression predisposes lymphocytes, particularly $CD4^+$ T cells, $CD8^+$ T cells, and antigen-presenting cells, to evolve into effectors of apoptosis and as a result, to lead to the destruction of healthy, non-infected T cells. Tat and Nef, along with Vpu, can also protect HIV-infected cells from apoptosis by increasing anti-apoptotic proteins and down-regulating cell surface receptors recognized by immune system cells. This review will discuss the validity of the apoptosis hypothesis in HIV disease and the potential mechanism(s) that HIV proteins perform in the progressive T cell depletion observed in AIDS pathogenesis.

### Keywords

HIV; AIDS; T cell depletion; apoptosis

## Introduction

Human immunodeficiency virus (HIV), a member of the primate lentivirus family of retroviruses, is the etiologic agent of AIDS. AIDS has become the major cause of death in individuals 25 to 44 years of age in the United States.[1] As of December 1999, 16.3 million people have died worldwide since the first cases were identified in 1981 and the total number of people living with HIV/AIDS is currently estimated at 35 million.[1] At the present rate, HIV is spreading faster in the human population than any infectious agent in the last 100 years.

HIV has become the most intensely studied infectious agent in history and the research generated has contributed to our increased understanding to several other fields of biology including virology, immunology, oncology, and cell biology. The primary receptor for these viruses is CD4 which was identified early in the studies of HIV pathogenesis.[2] CD4 is a necessary, but not a sufficient component for HIV-1 entry and replication.[3,4] Chemokine receptors, in conjunction with CD4, mediate the efficient entry of HIV into cells[5–9] and the use of different chemokine receptors by HIV accounts for the distinct cell tropism associated with various isolates.

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The viral replication cycle of HIV is highly regulated by viral gene products. The HIV-1 genome encodes the Gag, Pol, and Env structural proteins and two regulatory gene products, Tat and Rev. In addition, there are a number of accessory proteins encoded in the HIV-1 genome (Nef, Vif, Vpr, and Vpu) that are dispensable for *in vitro* replication but necessary to varying degrees for pathogenesis. As common for all retro-viruses, attachment of the HIV virion to specific receptors (CD4 and coreceptors) is followed by penetration of the core into the cell.[3,4] After entry, the single stranded RNA genome is converted into a double stranded DNA molecule by the viral RNA-dependent DNA polymerase, reverse transcriptase (RT). The proviral DNA form is transported to the nucleus and it integrates into host chromosomes, where synthesis of viral RNA is initiated and regulated by both cellular and viral proteins (Tat and Rev). Structural proteins (Gag-Pol and Env) are assembled and the viral genomic RNA is encapsidated into the newly synthesized virion. Post budding and processing, the new virions are released from the infected cell.

## AIDS, T cell depletion, and the apoptosis hypothesis

HIV-1 infection results in the progressive destruction of $CD4^+$ T lymphocytes. The pathogenic importance of the loss of these T cells correlates with disease progression and increases opportunistic infections, which subsequently leads to AIDS.[10] The mechanisms of $CD4^+$ T cell depletion during HIV infection are incompletely understood, however, this depletion can be mediated directly by HIV infection as a consequence of viral gene expression or indirectly through priming for apoptosis of uninfected 'bystander' cells.[10]

The mechanism of T cell-induced death involves the complimentary proteins Fas, also known as CD95 or Apo-1 and Fas ligand (Fas-L).[11–14] The ligation of Fas/Fas-L is the main mechanism for maintaining the appropriate number of T cells in the body. Therefore, this apoptotic pathway has been hypothesized to enhance HIV-1 expression in infected patients and subsequently to disrupt T cell homeostasis that eventually leads to the depletion of $CD4^+$ T lymphocytes. Several lines of evidence support this hypothesis. First, the proportion of Fas expressing T cells in HIV-1-infected individuals increase with disease progression.[15,16] Second, Fas-L message can be detected in freshly isolated HIV-infected T cells.[17] Third, therapies with anti-Fas antibody and soluble Fas-L induce T cells to undergo apoptosis.[18] In addition, induction of Fas message in peripheral blood lymphocytes (PBL), particularly in T cells and monocytes, from uninfected people can be upregulated when cocultured with HIV infected cells.[19] The significance of this last finding in terms of HIV-1 pathogenesis is unclear and has remained controversial. Other researchers have generated data to indicate that Fas-dependent activation does not induce T cell death in HIV-1-infected patients.[20,21]

This hypothesis, that apoptosis is important for HIV pathogenesis, has been proposed by several researchers.[22–26] A better understanding of HIV-mediated apoptosis is critical for the development of anti-retroviral therapies and vaccines. In this review, we will discuss the importance of apoptosis in HIV-1 disease and the role that HIV viral proteins contribute to the regulation of apoptosis.

## Endogenous and exogenous regulators of apoptosis

Apoptosis is an ordered, suicide mechanism that is characterized by cell shrinkage, loss of membrane integrity, chromosomal condensation, and internucleosomal cleavage of DNA. These changes are associated with the activation of cellular nucleases resulting in the fragmentation of DNA into a ladder of regular nucleosomal subunits.[27] It is now widely appreciated that apoptosis contributes to most types of physiological cell death. However, its participation in the pathological forms of cell death by HIV and other viruses is less fully understood and might vary depending on the particular pathological agent.

Studies analyzing the mechanisms responsible for apoptosis in human cells have revealed that the cysteinyl aspartate-specific protease (caspase) family constitutes the effector arm of apoptosis.[28–31] Caspases are synthesized as inactive proenzymes that are activated by upstream proteins in response to an apoptotic stimulus and are characterized by a cysteine located within their active site.[32] Following activation, caspases recognize specific sequences within their various target proteins and characteristically cleave these proteins 3′ of an aspartic acid residue within the recognition sequence.[33] Thus far, the 13 caspases identified differ with regard to the activation stimuli and to their substrate specificity and sensitivity to various inhibitors.[34,35] In addition to caspases, other apoptosis-inducing proteases can be activated in response to certain stimuli. One of these proteases, calpain, is a member of the calcium-dependent cysteine proteases and is involved in some forms of apoptosis.[36] Calpain substrates are primarily calmodulin-binding proteins.[36]

The idea that apoptosis could be used by the host immune system as a defense against viruses has been recognized for many years. A major line of defense against viral infections are cytotoxic T cells (CTL), which can recognize and induce apoptosis in virally infected cells.[37] CTL use more than one killing pathway: (1) CTL express the ligand for Fas and thus can kill Fas-bearing target cells; and (2) CTL bear granules that they can deliver into their target cells. One of the proteases in these CTL granules, granzyme B, cleaves after aspartate residues and activates many members of the caspase family.[37–40]

There are several proteins encoded by human cells and viruses that share structural similarities and can both promote (Bax, Bak, Bok) and inhibit (Bcl-2, Bcl-x) apoptosis.[40–45] These proteins are members of the *bcl-2* gene family and share sequence and functional homology to *Ced-9* of *Caenorhabditis elegans*.[46] The extensively studied Bcl-2 gene product is a major anti-apoptotic regulator and therefore, Bcl-2 itself is highly regulated. Cell-type specific expression of Bcl-2 and its interactions with the apoptotic protagonists, Bax and Bak are two major regulatory mechanisms of this protein.[47] Several members of this family dimerize via the Bcl-2 homology (BH) domain. Different sets of dimers either inhibit apoptosis (such as Bcl-2/Bax heterodimers) or induce apoptosis (such as Bax homodimers). The Bcl-2 member proteins that promote cell survival inhibit apoptosis upstream of caspase activation.[47,48]

A large number of viruses have evolved to encode specific inhibitors of caspase activation and apoptosis. Cytokine response modifier A (CrmA) from cowpox virus is similar to the serpin family of proteins and is able to inhibit caspase activation. CrmA acts as a substrate for caspase-1 and interferes with the host immune response to the virus.[49] The viral protein p35 (encoded by baculovirus) is a broad-spectrum inhibitor of apoptosis by acting as a substrate for several caspases.[50–52] To date, p35 blocks the induction of apoptosis by all stimuli tested.[51,52] Mutation of these genes allows for host cells to respond to infection by undergoing apoptosis that in turn drastically reduces viral spread. A number of adenoviruses encode the protein, E1B, which has similar properties to Bcl-2 by blocking apoptosis and interacting with Bak and Bax.

However, not all data support the important role of Bcl-2 or Fas/Fas-L interaction in HIV-1-induced disease. A recent study showed that during HIV-1 infection of T cells, E1B of adenovirus and Bcl-2 only marginally decreased overall cell death.[53] In addition, inhibition of Fas/Fas-L interaction did not prevent HIV-induced cell death.[53] Therefore, a non-apoptotic pathway may serve as a major pathway of HIV-induced cell death.

## Modulation of apoptosis by HIV infection

Primate retroviruses such as HIV-1, HIV-2, simian immunodeficiency virus (SIV), and human T cell leukemia virus (HTLV), have considerably smaller genomes compared to other viruses (herpesviruses, poxviruses, and baculoviruses) that encode genes for the regulation of

apoptosis. Nevertheless, cells infected with HIV have a prolonged survival time initiated by a variety of mechanisms including the modulation of Bcl-2, Fas, Fas-L, tumor necrosis factor α (TNFα) and caspase expression (Table 1).[54] HIV-1 is therefore not unique among viruses in regard to its ability to regulate apoptosis. HIV integrates into the host genome as part of its life cycle and, in general, integrated viruses that constitutively express viral gene products serve as good targets for the cellular immune response.[3,4] HIV-induced apoptosis may therefore play an important role in the viral life cycle by limiting the host immune response to virus infection and thus facilitating viral persistence.[55] HIV infection has been reported to abnormally activate T cells and change the balance between T helper (Th)-1 and Th2 cells, possibly by increasing apoptosis and decreasing $CD4^+$ T cells.[56] The modulation of apoptosis in HIV-infected and uninfected cells is complex. Figure 1 describes the overall integrated effect of various HIV gene products on apoptosis. Several viral proteins have been shown to effect apoptosis including Tat, Env, Nef, Vpr and Vpu (Table 1). The following sections address the specific actions of each of these proteins on the induction or inhibition of apoptosis in HIV-1 pathogenesis.

## Tat

Tat is expressed early after HIV infection and stimulates viral gene expression by interacting with the TAR element found in HIV mRNA.[57,58] Tat has been associated with several intracellular and extracellular proteins which suggests that Tat is capable of modulating various biological effects.[58] A soluble form of Tat (sTat) has been detected in HIV-1-infected patients and in the supernatant of Tat transfected cells, which can be taken up by neighboring cells.[59] Several reports indicate that Tat may effect B lymphocyte differentiation, growth of Kaposi sarcomas, the down-regulation of MHC class I and the induction and protection from apoptosis.[60–65] Even though the role of direct cell killing of $CD4^+$ T cells is a credible hypothesis for the T cell depletion seen in AIDS patients,[10] apoptosis is rarely observed in HIV-1-infected cells. Co-culture experiments indicate that while uninfected T cells die by apoptosis, HIV-infected cells remain resistant and this resistance has been partially mapped to *tat*.[55,66] Therefore, a dual role for Tat in regulation of apoptosis has been suggested: (1) Tat induces apoptosis of HIV uninfected cells via exogenous sTat by sensitizing bystander cells to Fas-mediated apoptosis; and (2) protecting HIV-infected cells from death by up-regulating T cell growth factors and anti-apoptotic proteins.[55]

Support for the 'bystander'-induced apoptosis theory is supported by experiments examining the lymph nodes of SIV-infected macaques.[55] Apoptosis occurs in the lymph nodes of these animals primarily in neighboring, uninfected cells and not in the productively infected T cells.[54] Exogenous sTat interacts with cell surface receptors, possibly CD26 and integrin α5β1, both of which can transduce signals leading to the down-regulation of Bcl-2 and induce apoptosis.[60,67,68] Tat induces Fas/Fas-L-dependent activation of apoptosis in T cell death. These findings indicate that Tat, by initiating signals in T cells, either directly or indirectly induces apoptosis in uninfected cells.[67] The Tat-induced signaling pathway has yet to be elucidated, but may involve the activation of inositol triphosphate (IP3)-phospholipase C (PLC) pathway.[69] Several proteins, including $p56_{lck}$, CD45, and hematopoietic cell phosphatase (HCP) as well as the transcription factors NF-κB and SP-1, have also been implicated in Tat-mediated apoptosis.[62]

In contrast to sTat, endogenous Tat appears to protect T lymphocytes from apoptosis. However, the mechanism for this protection remains unknown. *In vitro* studies indicate that cells transfected with Tat expression plasmids were resistant to apoptosis.[55] Endogenous Tat may block apoptosis by up-regulation of IL-2 or the anti-apoptotic proteins *bcl-2/bcl-x*.[70] Interestingly, endogenous Tat protects cells from exogenous sTat-induced apoptosis.

Tat can have pronounced immunosuppressive affects. In mice transfected with Tat expression vectors and in seroconverted infected humans, Tat expression cripples the immune surveillance of the infected host.[62–65,71–74] This immunosuppression affects both T cells and macrophages and can be reversed with Fas/Fas-L antagonists.[74] These observations have immediate relevance to HIV vaccine development. Several vaccine approaches for limiting HIV infection are currently underway and many include a Tat component in the vaccine. Vaccines in clinical and pre-clinical trials have included: (1) Tat expression vectors (both soluble and endogenous forms of Tat);[75,76] (2) as part of a multiple DNA expression plasmids encoding for several HIV genes;[77] (3) in live attenuated HIV vaccines;[78–83] and (4) in virus-like particles (VLPs) both in DNA expression vectors and as purified protein.[84] Therefore, a better comprehension of Tat-induced apoptosis and immunosuppressive mechanisms are important for new vaccine designs.[85] Interestingly, a recent study demonstrated that an oxidized, inactivated form of Tat does not produce the immunosuppressive effects associated with Tat and has been proposed for vaccinations.[86]

## Vpr

The 96 amino acid, 14 kDa accessory protein Vpr is highly conserved in HIV-1, HIV-2, and SIV and confers rapid growth advantage to viruses expressing this gene product.[87,88] Viruses expressing Vpr have faster growth rates than Vpr-deficient viruses. Also, the effects of Vpr are cell-type specific. Vpr augments the growth of HIV in macrophages, but does not enhance viral growth in primary T cells.[89] Vpr associates with the viral particle through the p6 molecule of Gag[90–92] and deletions or truncations of p6 prevent Vpr incorporation into the virion.[93] The incorporation of Vpr into progeny virions suggests that this protein participates in the early events of viral infection. Several functional roles have been demonstrated for Vpr during HIV replication. One of the more defined functions of Vpr is the nuclear translocation of the preintegration complex.[91,94–96] Vpr may enhance virus-specific functions such as reverse transcriptase by either stabilizing the RNA–DNA or DNA–DNA molecules, migration of the proviral DNA complex to the nucleus, or integration.[97] It has been shown that Vpr, along with the matrix p17 protein, acts to ensure the efficient nuclear import of the preintegration complex in non-dividing cells such as macrophages.[87]

Besides the role in nuclear translocation, *in vitro* functions have been described for Vpr including the enhancement of viral gene transcription.[92] Vpr is localized primarily in the nucleus. In dividing cells, Vpr has been shown to modestly *trans*-activate the HIV long terminal repeat (LTR) and heterologous viral promoters.[87,98] The exact mechanism by which Vpr increases protein synthesis is not clear,[99] however, it may be achieved by direct binding of Vpr to the transcription factors TFIIB and Sp1.[99,100]

In proliferating T cells and stimulated peripheral blood mononuclear cells (PBMCs), Vpr appears to be dispensable for the *in vitro* replication of HIV.[101–107] However, under certain conditions, Vpr contributes substantially to HIV-1 replication in these cells.[99] Vpr has been shown to induce cell cycle arrest of cells in the G2 phase of the cell cycle.[103,104,108,109] The Cdc2 kinase is an inactive form in G2-arrested cells. Recent reports suggest that Vpr disrupts the regulation of Cdc2 kinase, which leads to G2 arrest.[104,105,109] Vpr interacts with highly conserved components of the cell cycle regulation pathways. Interestingly, others have reported that Vpr specifically binds to the highly conserved DNA repair enzyme uracil DNA glycoslyase (UNG), that is involved in removing uracil from DNA. However, the role of UNG-Vpr interactions and cell cycle arrest is unclear.[110–112]

The different Vpr functions have been attributed to different regions of the protein.[88,91] The amino terminus is involved in virion incorporation and the nuclear location of the protein. The carboxyl terminus of the protein is required to induce G2 arrest. Analysis of Vpr point mutants

demonstrates a correlation between the extent of G2 arrest and the levels of apoptosis.[106] Mutants that cause more cells to accumulate in G2 phase of the cell cycle show increased levels of apoptosis.[109] However, Vpr-induced apoptosis does not require maintenance of G2 arrest. Studies by Stewart *et al,* indicate that treatment of Vpr-arrested cells with methlyxanthine pentoxifyllin alleviates G2 arrest but does not reduce the levels of apoptosis.[106] The Cdc2 kinase remained hyperphosphorylated during apoptosis, indicating that Cdc2 kinase was not required for activation of the apoptotic pathways induced by Vpr.

In contrast, reports by Bartz *et al*[104] show that Vpr induces cytostasis but not apoptosis. Infection of Jurkat T cells with virions expressing Vpr resulted in G2 arrest but not apoptosis.[104] These infected cells continued to accumulate at G2 over a 72 h period, however, no significant cell death was observed compared to Vpr mutant virions. The discrepancy between these two studies is mostly likely due to the post-infection time point at which the cells were monitored. Specifically, Bartz *et al*, monitored infected cells for only 3 days, whereas Stewart *et al*, observed that apoptosis increased over time and was usually maximal 4 days post-infection. Vpr increases apoptosis without addition of a second stimulus and Vpr-induced apoptosis has been shown to be independent of Cdc2 kinase activity.[106] Therefore, Vpr may contribute to the overall induction of cell death and contribute to $CD4^+$ T cell decline in disease via G2 arrest and subsequent induction of apoptosis.

## Env

Although the identification of specific coreceptors for HIV-1 infection represented an important step forward in our understanding of HIV-1 pathogenesis, information about how these receptors participate in the HIV replication cycle is just beginning to be understood. HIV infection requires binding of the gp120 subunit of Env to both CD4 receptor and one of a variety of chemokine receptors.[5,113] The chemokine receptor CXCR4 is required for entry of T-tropic isolates of HIV (named X4 strains) and CCR5 was determined to be the major coreceptor for M-tropic strains of HIV (R5 strains).[5–9,113] Even though these receptors are necessary for entry of HIV into cells, the importance of Env interaction with these receptors, resulting in signal transduction, is not totally understood. Activation of chemokine receptors by their cognate ligands or Env proteins results in prominent changes in cell migration and cell growth by stimulating various signal transduction pathways. Recent studies indicate that β-chemokines (which bind to CCR5) may exert indirect selective pressure *in vivo* in favor of the replication of X4 isolates that do not require CCR5 for entry.[114] Interestingly, this effect is evident only at low inoculum of HIV.[114] β-chemokine-mediated enhancement of X4 HIV replication can be detected at the early stages of HIV replication cycle and is dependent on signaling through G proteins. These findings provide insight regarding signals, generated from HIV Env receptors, which can enhance viral replication, possibly including signals that activate apoptosis. In that regard, Env from X4 strains of HIV can induce apoptosis *in vitro* in $CD8^+$ T cells and neuronal cells.[115,116] The signaling pathway induced by Env/CXCR4 interaction is complex, involving cross-talk with macrophages, and operates through the tumor-necrosis factor-α (TNF-α) receptor II (TNFRII).[114] Signaling by CXCR4 induces the surface expression of TNF-α in macrophages and TNFRII in $CD8^+$ T cells.[117–119] Subsequently, contact between the macrophages and T cells triggers T cell death. Although a low percentage of $CD8^+$ T cells were apoptotic in these studies, the loss of viable cells was high. Interestingly, this Env-induced death of $CD8^+$ T cells through CXCR4 and TNF-α/TNFRII signaling resembles the Env-induced death of $CD4^+$ T cells through CD4 and Fas/Fas-L receptor signaling.[119] TNF-α/TNFRII and Fas/Fas-L belong to the same apoptosis-transducing pathway, which are involved in the physiological control of T cells.[120] Therefore, X4-Envs may induce premature cell death in both $CD4^+$ and $CD8^+$ T cell populations through subverting signaling pathways that normally down-regulate critical immune responses.

The CD4 receptor itself has been shown to transduce signals after stimulation with HIV Envs leading to apoptosis.[121] One of the first studies to show the participation of apoptosis in $CD4^+$ T cells infected with HIV-1 *in vitro* was performed by Laurent-Crawford *et al.*[25] This study demonstrated that apoptosis was initiated in HIV-infected cells prior to cell lysis and suggested that apoptosis is the direct cause of cell death by HIV infection.[25] Also, stable cell clones expressing Env showed the characteristic features of apoptosis (DNA fragmentation and nuclear chromosome condensation) and these effects were seen as early as 8 h after induction.[86]

In addition to activating signaling by chemokine receptors, Env stimulates the induction of caspase-3 and caspase-6 in a CD4 receptor-dependent manner.[85,122] The activation of these two caspases is most likely the molecular mechanism responsible for the major apoptotic activity induced by the HIV envelope, since this activity can be inhibited by use of soluble CD4 (sCD4).[85,122] HIV Env also induces the phosphorylation of focal adhesion kinase (FAK) and the formation of this complex may contribute to the dysregulation of cellular activation and trafficking associated with HIV infection. FAK is cleaved in a caspase-3- and caspase-6-specific pattern and the cleavage of FAK by caspase-3 is associated with the early stages of apoptosis.[85,122] Thus, the activation and subsequent cleavage of phosphorylated FAK may represent the critical early step in HIV-1 induced apoptosis.

## Nef

Several biological activities have been attributed to the Nef protein of HIV, including the down-regulation of cell surface CD4,[123,124] and MHC class I,[122,123] enhancement of virion infectivity,[125–127] increased activation of expressing cells,[127,128] and enhancement of apoptosis.[129,130] *In vitro*, several researchers have reported difficulties in establishing stable cell lines constitutitvely expressing Nef due to Nef toxicity when this protein is expressed in transfected cell lines.[131,132]

The activation of apoptosis by Nef has recently been demonstrated by a variety of researchers. Okada *et al*[133] demonstrated that extracellular Nef induced apoptosis in both lymphoid and myeloid cells through a Fas-independent pathway. However, a contradictory study indicated that Nef induced Fas-L expression, which triggered Fas-mediated T cell death. Xu *et al*[134] showed that Nef and the ζ chain of the T cell receptor (TCR) were required and sufficient to upregulate Fas-L in T cells. Also lending support to this hypothesis, Fas-L activation in T cells by SIV depends on the expression of an intact Nef protein.[135] In addition, Nef from a highly pathogenic form of SIV (smmPBj14) alone can directly cause Fas-L up-regulation.[135]

Possible intracellular targets, important for Nef-mediated apoptosis, have been identified. Nef associates with cellular serine/threonine kinases (possibly PAK), as well as *src*-like tyrosine kinases (Hck and Lck).[136–138] Both of these types of kinases are important in T cell activation.[136–139] Consistent with this observation, Fas-L is expressed following activation of the TCR leading to the phosphorylation of these kinases and expression is inhibited by cell treatment with immunosuppressive drugs.[139] Ras signaling has also been implicated in tyrosine kinase activation and hence both the Ras and Lck pathways may contribute to the up-regulation of Fas-L transcription.[140] Therefore, HIV infection may activate tyrosine kinases (via Nef) that converge in a common activation pathway leading to Fas/Fas-L interaction and the induction of apoptosis.[129] Other HIV-1 gene products, particularly Tat and Env, together with Nef may act in concert to induce Fas-L and to increase Fas-based cytotoxicity.[19,141]

## Vpu

One of the least understood accessory protein expressed by HIV is Vpu. Two separate functions have been ascribed to Vpu. One of these functions is the ability of this protein to enhance HIV

release from infected cells.[26,142,143] This property is not restricted just to HIV, since Vpu can also enhance the release of virus particles generated from other retroviruses. A second function associated with Vpu is the disruption of Env–CD4 interactions in the endoplasmic reticulum.[26,142] Vpu appears to interact with the cytoplasmic tail of CD4 and targets CD4 to proteosomes for degradation. These two functions can be segregated to different regions of the Vpu protein.[26,142] The transmembrane region of Vpu is responsible for the increased viral release and the cytoplasmic tail is needed for CD4 degradation.[26,142]

Vpu has several structural similarities to the M2 protein of influenza virus.[143,144] Both M2 and Vpu form cation-selective ion channels.[145–147] The means by which Vpu ion channel activity might increase virus release is unknown. However, it is tempting to speculate that the ion channel properties of Vpu cause cells to be more responsive to apoptotic stimuli. Perturbations in potassium levels in neurons and decreased potassium levels in T cells directly cause apoptosis.[147–149] Interestingly, both Bcl-2 and Bax also have ion channel properties and may enhance ion-channel formation.[150–152]

Vpu has a large effect on the susceptibility of cells to Fas-induced apoptosis. Deletion of Vpu from the HIV genome increases survival in response to Fas cross-linking in both T cell lines and freshly isolated PBLs. In addition, an enhanced death rate is observed in cells infected with clones of HIV expressing Vpu. This enhanced rate correlates with increased Fas-induced cell death. However, deleting Vpu does not completely eliminate the sensitivity of HIV-1-infected cells to Fas killing. Most likely, the increase in cell death can be attributed to other viral gene products including Env and Tat. Interestingly, there is no homologue of Vpu in SIV or HIV-2. Therefore, the lack of Vpu in these two lentiviruses may help to explain their reduced pathogenicity compared to HIV-1. Interesting results have been obtained from rhesus macaques infected with SHIV chimeras (proviruses with an HIV *env* region contained in an SIV genomic backbone), which used a Vpu gene containing a mutated start codon (ACG).[153] Several months after infection, these monkeys developed AIDS-like symptoms. The virus recovered from these animals had a Vpu start codon that had reverted to the wild-type ATG codon and therefore Vpu was expressed in these animals,[153] again demonstrating that Vpu expression is important for inducing immunodeficiency disease.

## Summary

The regulation of apoptosis by HIV has profound implications for both the virus and the infected host cells. Induction of apoptosis has the potential of significantly influencing virus load and transmission rates as well as the response by the host immune system to the virus. HIV encodes a variety of gene products that can induce or inhibit apoptotic pathways (Figure 1). Apoptosis activation in bystander immune cells is the key to the depletion of T lymphocytes observed in HIV-1 infected patients. In a variety of model systems, viral genes can modulate apoptosis. Tat, Nef, and Vpu can protect infected cells from apoptotic stimuli. Env, Vpr, Vpu, and Tat can also induce apoptosis, particularly in non-infected cells. Understanding apoptotic mechanisms, induced by viral proteins, is important for the development of anti-HIV treatments. HIV-induced apoptosis may have detrimental effects on prophylactic administration of anti-viral drugs and vaccines. No vaccine capable of eliciting protective immunity to HIV infection has been formulated and HIV presents a formidable challenge to immune surveillance. The induction of apoptosis by HIV gene products, which could be expressed in a potential vaccine, may have adverse effects on the host. Therefore, a more thorough comprehension of the modulation of apoptosis by HIV proteins is critical for the development of an effective HIV vaccine and decreasing disease pathogenesis.

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**Figure 1.**

Model describing mechanisms of HIV modulation of apoptosis. HIV virions bind to cell surface CD4 and a coreceptor (primarily CCR5 or CXCR4) via Env initiating fusion with the cell membrane and releasing the virus-containing capsid into the cell cytoplasm. After reverse transcription process, the provirus is translocated to the nucleus and integrates into the host genome. Transcription of viral mRNA leads to the production of viral gene products. (1) Nef is expressed early in the life cycle and associates with the cell membrane where it interacts with a variety of proteins including p56lck. Nef interaction with the ζ chain of the TCR results in the up-regulation of Fas-L expression. (2) Fas-L expression can protect infected cells from cytotoxic T cell lymphocyte (CTL) killing by destroying immune cells expressing Fas. (3) Nef can down-regulate the expression of cell surface receptors CD4 and MHC class I and (4) protect the infected cells from recognition by CTLs. (5) Vpu can also prevent the cell surface expression of CD4 and therefore also protect infected cells from killing by CTLs. (6) In contrast to Nef and Vpu, Vpr expression can induce apoptosis by the induction of cell arrest at the $G_2$ phase of the cell cycle in HIV-infected cells. (7) Tat is among the first viral gene products to be expressed after proviral integration. Tat increases the expression of IL-2 (a T cell growth factor) and Bcl-2 (an anti-apoptotic protein), both of which protect infected cells from the induction of apoptosis. (8) However, expression of an exogenous, soluble form of Tat (sTat) induces apoptosis in many bystander cells. Interacting with cell surface receptors on T cells and macrophages, sTat can induce bystander cells to undergo apoptosis by initiating the expression of Fas, Fas-L, and TNF-α. In addition, sTat can down-regulate the expression of Bcl-2. Both TNF-α/TNFRII and Fas/Fas-L interactions can induce apoptosis in T cells by activation of caspases and the upregulation of Fas and Fas-L expression. (9) Progeny virion release can also induce apoptosis in bystander cells. The interaction of Env with cell receptors

initiates CD4 and CXCR4 cell signaling pathways and subsequently activates various caspases leading to cell death.

**Table 1**

HIV gene products involved in modulation of apoptosis

| *HIV protein* | *Mechanisms of action* | *Effect on apoptosis* |
|---|---|---|
| Env | Signal activation of CD4 | Enhancement |
| | Signal activation of chemokine receptor | Enhancement |
| | Syncytium formation | Enhancement |
| Nef | Activation of Fas-L expression | Enhancement |
| | Down-regulation of CD4 and MHC I | Inhibition |
| Tat | Signal transduction in Fas-dependent manner | Enhancement |
| | Activation of cyclin-dependent kinases | Enhancement |
| | Increases in Bcl-2 expression | Inhibition |
| | Increases in IL-2 expression | Inhibition |
| Vpr | Induction of cell cycle $G_2$ arrest | Enhancement |
| | Activation of IκB | Inhibition |
| Vpu | Increases in ion channel activity | Enhancement |
| | Interactions with Fas | Enhancement |
| | Down-regulation of CD4 | Inhibition |